# Revised Irradiation Doses to Control Melon Fly, Mediterranean Fruit Fly, and Oriental Fruit Fly (Diptera: Tephritidae) and a Generic Dose for Tephritid Fruit Flies

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**ABSTRACT** Currently approved irradiation quarantine treatment doses for *Bactrocera cucurbitae* (Coquillet), melon fly; *Ceratitis capitata* (Wiedemann), Mediterranean fruit fly; and *Bactrocera dorsalis* (Hendel), oriental fruit fly, infesting fruits and vegetables for export from Hawaii to the continental United States are 210, 225, and 250 Gy, respectively. Irradiation studies were initiated to determine whether these doses could be reduced to lower treatment costs, minimize any adverse effects on quality, and support a proposed generic irradiation dose of 150 Gy for fruit flies. Dose–response tests were conducted with late third instars of wild and laboratory strains of the three fruit fly species, both in diet and in fruit. After x-ray irradiation treatment, data were taken on adult emergence, and adult female fecundity and fertility. Melon fly was the most tolerant of the three species to irradiation, and oriental fruit fly was more tolerant than Mediterranean fruit fly. Laboratory and wild strains of each species were equally tolerant of irradiation, and larvae were more tolerant when irradiated in fruit compared with artificial diet. An irradiation dose of 150 Gy applied to 93,666 melon fly late third instars in papayas resulted in no survival to the adult stage, indicating that this dose is sufficient to provide quarantine security. Irradiation doses of 100 and 125 Gy applied to 31,920 Mediterranean fruit fly and 55,743 oriental fruit fly late third instars, respectively, also resulted in no survival to the adult stage. Results support a proposed generic irradiation quarantine treatment dose of 150 Gy for all tephritid fruit flies.

**KEY WORDS** x-ray irradiation, quarantine pest, phytosanitary treatment

FRUITS AND VEGETABLES GROWN in Hawaii are subject to federal quarantine regulations because of four exotic tephritid fruit flies—*Bactrocera cucurbitae* (Coquillet), melon fly; *Ceratitis capitata* (Wiedemann), Mediterranean fruit fly; *Bactrocera dorsalis* (Hendel), oriental fruit fly; and *Bactrocera latifrons* (Hendel), solanaceous fruit fly (Diptera: Tephritidae)—and other pests. Quarantine treatments such as heat, cold, irradiation, and fumigation disinfest host commodities of insect pests before they are exported to the U.S. mainland where the pests do not occur. Hawaii has approved quarantine treatments for 14 different tropical fruits and five vegetables (Follett 2004); irradiation is an accepted quarantine treatment to control fruit flies in 10 fruits and five vegetables exported from Hawaii (Federal Register 2003). Whereas development of heat and cold treatments involves generating data for each fruit host and pest combination, irradiation treatments are developed for a pest species irrespective of fruit host. This is possible because most commodities can tolerate irradiation at doses that kill the pest, whereas developing heat and cold treatments involves finding a balance between killing the pest and minimizing the adverse effects of the treatment process on commodity quality (Moy and Wong 2002, Follett and Sanxter 2003).

A "generic" quarantine treatment is one that provides quarantine security for a broad group of pests. For example, a generic treatment could be applied to all Diptera (flies), or to flies in the family Tephritidae (fruit flies), or to tephritid fruit flies in the genus *Bactrocera.* Irradiation is the ideal technology for developing generic treatments because it is effective against most insects and mites at dose levels that do not affect the quality of most commodities. Before generic treatments can be recommended, information is needed on effective irradiation doses for a wide range of insects within the taxon.

The International Consultative Group on Food Irradiation (ICGFI) was the first group to formalize a recommendation for generic irradiation treatments (ICGFI 1991). In 1986, based on irradiation data for several tephritid fruit fly species and a limited number of other insect pests, they proposed a dose of 150 Gy for fruit flies and 300 Gy for other insects. To date, these doses have not been adopted, partly because earlier research suggested that three tephritid fruit fly species in Hawaii required higher irradiation doses to

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prevent adult emergence from infested fruit. Based on results of Seo et al. (1973), the USDA–Animal Plant Health Inspection Service (APHIS) approved irradiation doses of 210, 225, and 250 Gy for melon fly, Mediterranean fruit fly, and oriental fruit fly, respectively, for exporting fruits and vegetables from Hawaii (Federal Register 1997).

Before adopting a generic dose for tephritid fruit flies, further irradiation studies were needed with melon fly, Mediterranean fruit fly, and oriental fruit fly in Hawaii to determine whether the approved doses are unnecessarily high and could be reduced. Secondary objectives were to compare the radiotolerance of laboratory strain and wild fruit flies, and to determine the extent to which treatment substrate can modify radiotolerance.

## Methods

Comparative dose–response tests were conducted with wild and laboratory strains of the melon fly, Mediterranean fruit fly, and oriental fruit fly, both in diet and in fruit. The primary goal was to find irradiation treatments to prevent adult emergence, but reproductive performance of adults surviving treatment was also examined. Wild melon flies and oriental fruit flies were collected weekly from a papaya farm in Kapoho, HI, and reared on papaya. All irradiation tests were conducted with $F_1$ generation flies. Laboratory flies used in tests were obtained from colonies maintained at the USDA-ARS laboratory in Honolulu, HI, and were reared on standard diets for each species (Vargas 1989). Wild Mediterranean fruit flies were collected periodically from wild Jerusalem cherry, *Solanum pseudocapsicum* L., in Hawaii Volcanoes National Park, HI, and reared on papaya in the laboratory. The Mediterranean fruit fly laboratory strain was Maui hybrid strain 2000, started in 1997 from crosses between Maui-med strain males and wild coffee females (McInnis et al. 2002). Rearing conditions for all species for the duration of the experiments were 25 ± 2°C and a photoperiod of 14:10 (L:D) h.

Late third instars were used in all tests because this is the most radiotolerant life stage occurring in fruit for Hawaii's tephritid fruit flies (Balock et al. 1963). For each test, 150 larvae were introduced to the cavity at the center of a 'Kapoho solo' papaya (1/2–3/4 ripe) through a hole made with a 12-mm cork borer, or placed on standard larval rearing diet in a 0.24-liter plastic cup. Larvae were transferred to papaya or diet 24 h before irradiation to allow larvae to distribute themselves in the media. Irradiation treatment was conducted at a nearby commercial x-ray irradiation facility (Hawaii Pride, Keaau, HI) by using an electron linear accelerator (5 MeV, model TB-5/15, SureBeam Corp., San Diego, CA). Detailed dose mapping was conducted before initiating the experiments (Follett and Lower 2000), and ROW dosimeters (Opti-chromic detectors, FWT-70-83M, Far West Technology, Goleta, CA) were placed in a representative fruit or diet tray at each dose in each replicate to measure dose variation. Dosimeters were read with an FWT-200 reader (Far West Technology, Goleta, CA) at 600-nm absorbance. Depending on the species, dose–response tests consisted of five to eight doses at 10-Gy increments within a range from 20 to 110 Gy. The dose uniformity (or maximum-minimum) ratio was ≤1.2 for all tests. Tests usually included 300 larvae per dose (150 larvae in each of two fruit, a third fruit with a dosimeter), replicated four to eight times, and in each replicate a control group was not irradiated. Wild Mediterranean fruit flies from Jerusalem cherry did not survive well during rearing on papaya in preliminary tests; therefore, irradiation dose–response tests included only 50 larvae per dose, replicated two to seven times, and rearing methods were modified to minimize handling and reduce control mortality. After irradiation treatment, papayas or diet containing larvae was placed in screened 3.8-liter plastic tubs (Rubbermaid, Wooster, OH) over sand. Sand was sifted at weekly intervals to remove developing pupae and transfer them to 0.24-liter plastic cups. Development of all individuals in a test was followed until death while recording adult emergence and testing female fecundity and fertility.

To test reproductive performance of flies surviving irradiation treatment, 25 females flies were randomly selected at each dose and replicate, and transferred from mating cages (male and female flies) to 0.5-liter plastic tubs containing a 4.0-cm-diameter disc of fresh papaya skin; the papaya skin disc was perforated ≈50 times with a #3 probe to facilitate oviposition. After 4 h, eggs were transferred from the papaya skin onto black filter paper (7 cm diameter) in a petri dish. Egg hatch was recorded after 72–96 h. For each treatment, egg collection was performed for three successive weeks after flies had begun laying eggs (typically 2–3 wk after emergence). When fewer than 25 females survived treatment, all live females available were used for each egg deposition test. Each replicate included an equal number of untreated control flies for comparison.

Large-scale confirmatory tests were conducted with each species. Large-scale tests usually involved irradiating ≈4,800 late third instars in papayas (as described above) at a single dose predicted to prevent adult emergence; this was repeated 7–15 times depending on the species, and each time a control group of 200–400 late third instars was not irradiated. Additional large-scale tests were conducted with melon fly by using natural oviposition in the laboratory to infest papayas rather than artificial inoculation. In quarantine research, the maximum irradiation dose applied in experimental testing is the minimum absorbed dose proposed for a certified treatment. Irradiation treatment levels were deliberately set so that the insects would receive a dose less than the target dose; however, in a few replicates treatment levels slightly exceeded the target dose.

To make comparisons between treatments, dose–response data were subjected to linear regression and analysis of covariance (ANCOVA) by using the standard least squares model. Data used in the linear regression model included any irradiation dose causing

**Table 1. Effects of irradiation on maturation of melon fly third instars**

| Strain | Substrate | Dose (Gy) | No. larvae treated | No. puparia recovered | Dead larvae recovered | Partial pupal emergence | Deformed adults | | Normal adults | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Males | Females | Males | Females |
| Wild | Fruit | 0 | 2,460 | 2,010 | 92 | 37 | 30 | 29 | 701 | 837 |
| | | 20 | 1,920 | 1,425 | 112 | 127 | 36 | 37 | 475 | 450 |
| | | 30 | 2,670 | 2,047 | 234 | 143 | 34 | 26 | 393 | 358 |
| | | 40 | 2,370 | 1,939 | 125 | 83 | 25 | 20 | 277 | 262 |
| | | 50 | 1,920 | 1,479 | 83 | 91 | 24 | 12 | 146 | 128 |
| | | 60 | 1,500 | 1,172 | 67 | 38 | 10 | 9 | 70 | 10 |
| | | 70 | 1,500 | 1,249 | 49 | 81 | 5 | 2 | 25 | 24 |
| | | 80 | 1,500 | 1,140 | 72 | 60 | 2 | 4 | 4 | 0 |
| | | 90 | 1,200 | 885 | 41 | 9 | 1 | 0 | 1 | 0 |
| | | 100 | 1,500 | 1,138 | 90 | 12 | 0 | 0 | 0 | 0 |
| Laboratory | Fruit | 0 | 1,200 | 1,099 | 8 | 31 | 13 | 6 | 364 | 389 |
| | | 40 | 1,200 | 1,037 | 33 | 20 | 7 | 3 | 123 | 94 |
| | | 50 | 1,200 | 1,073 | 8 | 44 | 5 | 3 | 116 | 89 |
| | | 60 | 1,200 | 1,095 | 28 | 71 | 4 | 2 | 52 | 36 |
| | | 70 | 1,200 | 1,069 | 23 | 59 | 3 | 1 | 37 | 13 |
| | | 80 | 1,200 | 1,042 | 17 | 11 | 0 | 0 | 5 | 2 |
| | | 90 | 1,200 | 1,097 | 17 | 1 | 0 | 0 | 0 | 0 |
| | | 100 | 1,200 | 1,035 | 24 | 1 | 0 | 0 | 0 | 0 |
| Laboratory | Diet | 0 | 1,200 | 1,144 | 2 | 3 | 2 | 1 | 567 | 531 |
| | | 20 | 1,200 | 1,153 | 16 | 217 | 47 | 23 | 304 | 225 |
| | | 30 | 1,200 | 1,152 | 13 | 26 | 3 | 3 | 19 | 6 |
| | | 40 | 1,200 | 1,133 | 18 | 4 | 1 | 0 | 10 | 10 |
| | | 50 | 1,200 | 1,170 | 7 | 0 | 0 | 0 | 1 | 0 |
| | | 60 | 1,200 | 1,165 | 5 | 0 | 0 | 2 | 1 | 1 |
| | | 70 | 1,200 | 1,165 | 3 | 1 | 0 | 0 | 0 | 0 |
| | | 80 | 1,200 | 1,165 | 15 | 1 | 0 | 0 | 0 | 0 |

mortality between 0 and 100%, and the lowest dose causing 100% mortality. For each replicate, mortality values <100% were adjusted for control mortality using Abbott's formula (Abbott 1925). Percentage mortality data were arcsine transformed to help normalize the distribution before statistical analysis (SAS Institute 2002). Residual plots were evaluated to ensure regression model assumptions were met for each treatment combination. Covariance analysis requires the slopes of the regression lines fitted to each group to be parallel, so the assumption of parallelism (nonsignificant treatment × dose interaction effect) was tested before comparing intercepts (treatment effects) (Sokal and Rohlf 1981). The effects of irradiation dose on prevention of adult emergence was compared 1) among the three fruit fly species irradiated in fruit (species effect), 2) between wild and laboratory strains (strain effect), and 3) between laboratory strains irradiated on diet and in fruit (substrate effect). Linear regression and analysis of variance (ANOVA) on transformed data were used to compare species, strains, and substrates rather than standard nonlinear models (logit or probit) because of the limited number of points and better fit to the data. Data were plotted using Sigma Plot (1997).

## Results

Emergence of normal adults from irradiated third instars decreased with increasing dose for all species, strains, and substrates (Tables 1–4). The lowest irradiation dose showing no adult survival varied by species and substrate. One melon fly adult emerged at 90 Gy (Table 1), whereas the highest doses with survivors for Mediterranean fruit fly and oriental fruit fly and were 50 Gy (Tables 2 and 3) and 70 Gy (Table 4), respectively. Survivorship was lower for fruit flies irradiated on diet compared with in fruit. For laboratory strain melon flies, two adults emerged from 1,200 larvae irradiated at 60 Gy on diet, whereas 88 adults emerged from 1,200 larvae irradiated at this dose on papaya. Likewise, for laboratory strain oriental fruit flies, no adults emerged from 1,230 larvae irradiated at 40 Gy in diet, whereas 75 adults emerged from 900 larvae irradiated at this dose on papaya. Irradiation did not affect pupariation rates in any treatment.

**Species Differences.** The three fruit fly species differed in their tolerance of irradiation. For wild strain flies reared and irradiated in fruit, species by dose interaction effects were not significantly different between melon fly and Mediterranean fruit fly ($F = 0.18$; df = 1, 3; $P = 0.67$), melon fly and oriental fruit fly ($F = 0.02$; df = 1, 3; $P = 0.88$), and oriental fruit fly and Mediterranean fruit fly ($F = 0.05$; df = 1, 3; $P = 0.82$), indicating that slopes of the regression lines were parallel and therefore intercepts (species effects) could be compared (Fig. 1). The effect of species was highly significant for all pairs of wild fly species; melon

**Table 2. Effects of irradiation on maturation of wild strain Mediterranean fruit fly third instars**

| Strain | Substrate | Dose | No. larvae treated | Partial pupal emergence | Deformed adults | Normal adults |
|---|---|---|---|---|---|---|
| Wild | Fruit | 0 | 350 | 0 | 5 | 174 |
| | | 20 | 200 | 0 | 2 | 12 |
| | | 30 | 300 | 0 | 1 | 6 |
| | | 40 | 350 | 0 | 1 | 3 |
| | | 50 | 100 | 0 | 0 | 0 |
| | | 60 | 100 | 0 | 0 | 0 |

**Table 3. Effects of irradiation on maturation of laboratory strain Mediterranean fruit fly third instars**

| Strain | Substrate | Dose (Gy) | No. larvae treated | No. puparia recovered | Dead larvae recovered | Partial pupal emergence | Deformed adults | | Normal adults | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Males | Females | Males | Females |
| Laboratory | Fruit | 0 | 1,500 | 1,299 | 35 | 26 | 4 | 3 | 520 | 607 |
| | | 20 | 1,500 | 1,344 | 25 | 24 | 3 | 3 | 44 | 52 |
| | | 25 | 1,350 | 1,138 | 22 | 16 | 2 | 4 | 17 | 37 |
| | | 30 | 1,500 | 1,345 | 38 | 18 | 2 | 2 | 9 | 14 |
| | | 35 | 1,050 | 985 | 7 | 4 | 0 | 1 | 2 | 4 |
| | | 40 | 1,500 | 1,307 | 12 | 7 | 0 | 0 | 1 | 6 |
| | | 45 | 1,200 | 1,058 | 8 | 4 | 0 | 0 | 1 | 2 |
| | | 50 | 1,500 | 1,396 | 15 | 2 | 0 | 0 | 0 | 1 |
| | | 60 | 300 | 282 | 7 | 0 | 0 | 0 | 0 | 0 |
| | | 70 | 300 | 272 | 4 | 0 | 0 | 0 | 0 | 0 |
| | | 80 | 300 | 286 | 2 | 0 | 0 | 0 | 0 | 0 |
| | Diet | 0 | 1,800 | 1,704 | 44 | 19 | 17 | 9 | 770 | 622 |
| | | 15 | 3,300 | 3,082 | 83 | 119 | 47 | 47 | 337 | 286 |
| | | 20 | 1,500 | 1,347 | 37 | 8 | 2 | 2 | 21 | 23 |
| | | 25 | 1,500 | 1,436 | 35 | 2 | 1 | 3 | 13 | 8 |
| | | 30 | 1,500 | 1,375 | 18 | 0 | 0 | 1 | 0 | 1 |
| | | 35 | 1,500 | 1,424 | 32 | 0 | 0 | 0 | 0 | 0 |
| | | 40 | 1,200 | 1,128 | 31 | 0 | 0 | 0 | 0 | 0 |

fly was significantly more tolerant of irradiation than Mediterranean fruit fly ($F = 27.1$; df = 1, 3; $P = 0.0001$) and oriental fruit fly ($F = 24.9$; df = 1, 3; $P = 0.0001$), and oriental fruit fly was more tolerant of irradiation than Mediterranean fruit fly ($F = 15.6$; df = 1, 3; $P = 0.0003$).

For laboratory strain flies reared and irradiated in fruit, species by dose interaction effects were not significantly different for comparisons between melon fly and Mediterranean fruit fly ($F = 1.4$; df = 1, 3; $P = 0.25$) or oriental fruit fly ($F = 0.8$; df = 1, 3; $P = 0.38$), indicating slopes of regression lines were parallel and therefore intercepts (species effects) could be compared (Fig. 2). The species effect was significant for both pairs; melon fly was significantly more tolerant of irradiation than Mediterranean fruit fly ($F = 36.8$; df = 1, 3; $P = 0.0001$) and oriental fruit fly ($F = 7.1$; df = 1, 3; $P = 0.01$). The species by dose interaction effect was significant for the comparison between Mediterranean fruit fly and oriental fruit fly ($F = 7.7$; df = 1, 3; $P = 0.01$), indicating slopes were not parallel and so the species could not be compared (Fig. 2).

For laboratory strains of flies reared and irradiated on diet, species by dose interaction effects were not significant (at $P \leq 0.05$) for comparisons between melon fly and Mediterranean fruit fly ($F = 2.8$; df = 1, 3; $P = 0.10$), melon fly and oriental fruit fly ($F = 3.45$; df = 1, 3; $P = 0.07$), and oriental fruit fly and Mediterranean fruit fly ($F = 0.01$; df = 1, 3; $P = 0.90$), indicating that slopes were approximately parallel and therefore intercepts (species effects) could be compared (Fig. 3). Species effects were highly significant

**Table 4. Effects of irradiation on maturation of oriental fruit fly third instars**

| Strain | Substrate | Dose (Gy) | No. larvae treated | No. puparia recovered | Dead larvae recovered | Partial pupal emergence | Deformed adults | | Normal adults | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Males | Females | Males | Females |
| Wild | Fruit | 0 | 1,190 | 1,043 | 44 | 9 | 5 | 6 | 450 | 359 |
| | | 20 | 1,320 | 938 | 108 | 17 | 13 | 18 | 200 | 239 |
| | | 30 | 1,980 | 1,297 | 177 | 17 | 16 | 8 | 110 | 114 |
| | | 40 | 1,260 | 919 | 177 | 5 | 8 | 6 | 33 | 30 |
| | | 50 | 886 | 632 | 128 | 4 | 3 | 5 | 25 | 34 |
| | | 60 | 886 | 675 | 114 | 6 | 7 | 4 | 14 | 13 |
| | | 70 | 870 | 599 | 156 | 1 | 1 | 0 | 6 | 1 |
| | | 80 | 890 | 633 | 122 | 1 | 0 | 0 | 0 | 0 |
| | | 90 | 300 | 196 | 45 | 0 | 0 | 0 | 0 | 0 |
| Laboratory | Fruit | 0 | 900 | 795 | 9 | 11 | 3 | 4 | 370 | 335 |
| | | 20 | 1,500 | 1,276 | 16 | 25 | 11 | 9 | 229 | 221 |
| | | 30 | 1,200 | 1,074 | 8 | 28 | 12 | 13 | 156 | 163 |
| | | 40 | 900 | 732 | 6 | 6 | 6 | 6 | 36 | 39 |
| | | 50 | 600 | 591 | 0 | 25 | 10 | 11 | 33 | 37 |
| | | 60 | 600 | 514 | 2 | 7 | 0 | 0 | 2 | 0 |
| | | 70 | 600 | 516 | 7 | 1 | 0 | 0 | 0 | 0 |
| | | 80 | 600 | 530 | 8 | 0 | 0 | 0 | 0 | 0 |
| Laboratory | Diet | 0 | 1,103 | 1,097 | 5 | 4 | 3 | 1 | 543 | 503 |
| | | 15 | 1,208 | 1,200 | 8 | 49 | 63 | 67 | 221 | 221 |
| | | 20 | 1,223 | 1,180 | 11 | 31 | 23 | 37 | 72 | 53 |
| | | 25 | 1,233 | 1,111 | 3 | 10 | 11 | 8 | 32 | 37 |
| | | 30 | 1,218 | 1,142 | 9 | 4 | 1 | 1 | 8 | 5 |
| | | 35 | 1,220 | 1,146 | 7 | 0 | 1 | 2 | 8 | 7 |
| | | 40 | 1,230 | 1,166 | 12 | 0 | 0 | 0 | 0 | 0 |

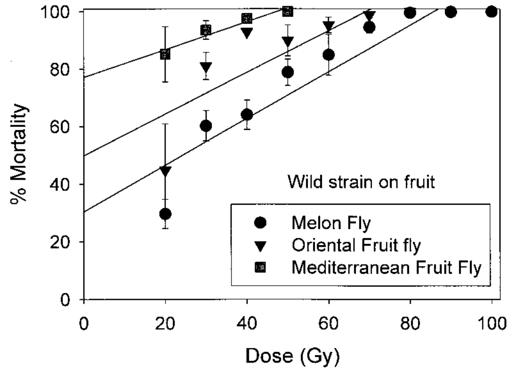


**Fig. 1.** Effect of irradiation dose on mortality (failure to emerge as adult capable of flight) (mean ± SE) of wild strain flies in papaya. Equations for the regression lines were y = 0.81x + 30.4 ($r^2$ = 0.86) for melon fly, y = 0.73x + 49.9 ($r^2$ = 0.66) for oriental fruit fly, and y = 0.48x + 77.2 ($r^2$ = 0.93) for Mediterranean fruit fly. Melon fly was the most tolerant of the three species to irradiation.

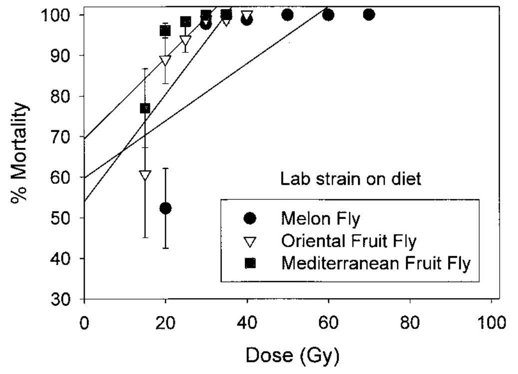


**Fig. 3.** Effect of irradiation dose on mortality (failure to emerge as adult capable of flight) (mean ± SE) of laboratory strain flies in diet. Equations for the regression lines were y = 0.70x + 59.7 ($r^2$ = 0.47) for melon fly, y = 1.32x + 54.0 ($r^2$ = 0.67) for oriental fruit fly, and y = 1.0x + 69.4 ($r^2$ = 0.65) for Mediterranean fruit fly. Melon fly was the most tolerant of the three species to irradiation.

for all pairs. Melon fly was significantly more tolerant of irradiation than Mediterranean fruit fly ($F$ = 23.2; df = 1, 3; $P$ = 0.0001) and oriental fruit fly ($F$ = 11.7; df = 1, 3; $P$ = 0.0001), and oriental fruit fly was more tolerant of irradiation than Mediterranean fruit fly ($F$ = 5.9; df = 1, 3; $P$ = 0.02).

**Wild versus Laboratory Strains.** All wild and laboratory flies used in this comparison were reared and irradiated in papaya fruit. Strain × dose interaction effects were not significant for comparisons between wild and laboratory strains of the melon fly ($F$ = 0.005; df = 1, 3; $P$ = 0.94), Mediterranean fruit fly ($F$ = 0.35; df = 1, 3; $P$ = 0.56), and oriental fruit fly ($F$ = 1.28; df = 1, 3; $P$ = 0.26) irradiated in papaya, suggesting slopes were parallel and therefore intercepts (strain effects) could be compared. Strain effects were not significant for any species; wild and laboratory strains of melon fly were equally tolerant of irradiation ($F$ = 0.82; df = 1, 3; $P$ = 0.37) as were laboratory and wild strains of Mediterranean fruit fly ($F$ = 1.34; df = 1, 3; $P$ = 0.26) and oriental fruit fly ($F$ = 0.01; df = 1, 3; $P$ = 0.94). These results with third instars suggest wild and laboratory strains of the three species can be used interchangeably in irradiation tests.

**Effect of Substrate.** The flies used in this comparison were laboratory strain flies reared on diet before irradiation and then treated and held in either of two substrates, papaya or diet. Substrate × dose interaction effects were not significant for melon fly ($F$ = 0.03; df = 1, 3; $P$ = 0.86) or oriental fruit fly ($F$ = 2.1; df = 1, 3; $P$ = 0.15), suggesting slopes were parallel and therefore intercepts (substrate effects) could be compared. The substrate by dose interaction effect was significant for the comparison between papaya and

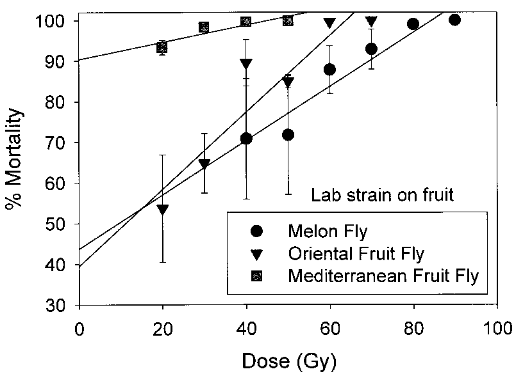


**Fig. 2.** Effect of irradiation dose on mortality (failure to emerge as adult capable of flight) (mean ± SE) of laboratory strain flies in papaya. Equations for the regression lines were y = 0.67x + 43.8 ($r^2$ = 0.92) for melon fly, y = 0.95x + 39.5 ($r^2$ = 0.87) for oriental fruit fly, and y = 0.21x + 90.4 ($r^2$ = 0.79) for Mediterranean fruit fly. Melon fly was the most tolerant of the three species to irradiation.

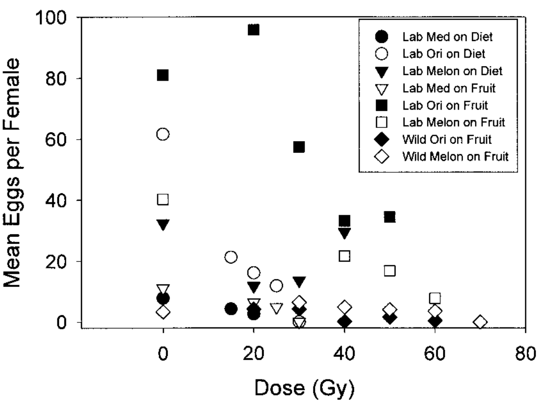


**Fig. 4.** Effect of irradiation dose on the number of eggs laid by laboratory and wild strains of Mediterranean fruit fly, oriental fruit fly, and melon fly treated as third instars on diet and in papaya. Med, Mediterranean fruit fly; Ori, oriental fruit fly; and Melon, melon fly.

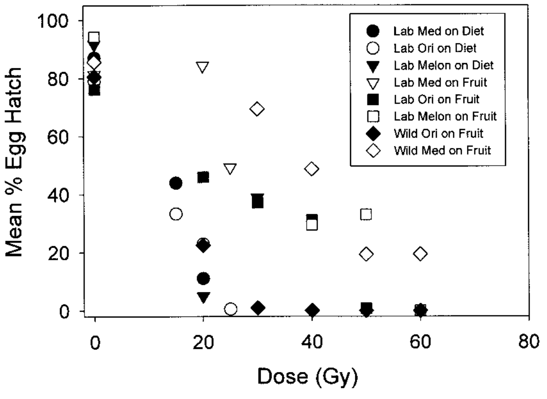


**Fig. 5.** Effect of irradiation dose on percentage of egg hatch of laboratory and wild strains of Mediterranean fruit fly, oriental fruit fly, and melon fly treated as third instars on diet and in papaya. Med, Mediterranean fruit fly; Ori, oriental fruit fly; and Melon, melon fly.

diet for Mediterranean fruit fly ($F = 10.3$; df = 1, 3; $P = 0.003$), indicating slopes were not parallel and therefore substrate effects were not compared. Melon fly was significantly more tolerant of irradiation when treated in fruit compared with diet ($F = 23.1$; df = 1, 3; $P = 0.0001$) as was oriental fruit fly ($F = 41.9$; df = 1, 3; $P = 0.0001$). These results emphasize the importance of irradiating insects in their natural host rather than on an artificial substrate.

**Effect on Reproduction.** The mean number of eggs per female (Fig. 4) and the mean percentage of hatch of eggs (Fig. 5) for all treatments generally decreased with increasing dose (all slopes were negative), but not all slopes were significantly different from zero. Slopes for the mean number of eggs per female were significant for laboratory strain oriental fruit flies on diet and in fruit ($P = 0.004$ and 0.005, respectively), laboratory and wild strain melon flies on fruit (both $P = 0.02$), and wild strain oriental fruit flies on fruit ($P = 0.03$). Slopes for percentage of egg hatch were significant for laboratory strain Mediterranean fruit flies on diet and fruit (both $P = 0.05$), and laboratory strain melon flies on fruit ($P = 0.009$). Egg deposition is inherently variable in tephritid fruit flies, and in some cases the increase in mortality with increasing irradiation dose resulted in only three doses having a sufficient number of adults emerging to conduct the mating assay. For example, 117 female laboratory strain oriental fruit flies irradiated at 15 Gy on diet were mated and available for egg deposition testing, but only 37 females at 25 Gy and three females at 30 Gy were available for testing. Although the number of eggs produced by flies seemed to be primarily a function of irradiation dose, it was also potentially a function of the number of flies available for mating. Also, laboratory and wild strains differed widely in their response to the oviposition bioassay. For example, untreated laboratory strain oriental fruit flies and melon flies on fruit produced a mean of 81.0 and 40.3 eggs per female, respectively, whereas wild strains produced 3.3 and 3.4 eggs per female, respectively. (Low reproductive performance is a common observation for wild tephritid fruit flies brought into the laboratory.) These problems made statistical comparisons among species, strains, and substrates impractical for the egg deposition and hatch data.

**Large-Scale Confirmatory Tests.** Melon fly was the most radiotolerant of the three species (Figs. 1–3). Predicted doses from dose–response data for 100% mortality (no adult emergence) were 85.7 (79.7–93.6 [95% CL]) Gy and 84.5 (72.9–121.0 [95% CL]) Gy for wild and laboratory strain melon fly third instars, respectively, irradiated on papaya. Dose–response data showed a few partially emerged melon fly pupae (puparium broken open but adult fly unable to fully emerge) at a dose of 100 Gy. Therefore, 120 Gy was initially selected for large-scale confirmatory testing with wild melon fly in papaya. One adult capable of flight survived when 47,800 melon fly late third instars were irradiated at a maximum absorbed dose of 124 Gy (Table 5), and several pupae were partially emerged. When the irradiation dose was increased to 150 Gy, treatment of 93,666 melon fly third instars resulted in zero survivors to the adult stage (Table 5), and no pupae were partially emerged. Adult emergence in controls was 57%. Irradiation of 31,920 Mediterranean fruit fly third instars in papaya at a maximum absorbed dose of 100 Gy resulted in 0 survivors to the adult stage (Table 5), and no pupae were partially emerged. Adult emergence in controls was 59%. Irradiation of 32,588 wild and 23,155 laboratory strain oriental fruit fly third instars in papaya at a maximum absorbed dose of 124 Gy (target dose of 120 was exceeded) resulted in zero

**Table 5. Large-scale confirmatory tests irradiating third instars of three fruit fly species in papaya**

| Species | Strain | Target dose (Gy)[a] | Measured doses (Gy) | No. tested[b] | No. survivors to adult |
|---|---|---|---|---|---|
| Melon fly[c] | Wild | 120 | 113–124 | 47,800 | 1 |
| | Wild | 150 | 129–144 | 93,666[d] | 0 |
| Mediterranean fruit fly | Laboratory | 100 | 73–100 | 31,920 | 0 |
| Oriental fruit fly | Wild | 120 | 90–118 | 23,155 | 0 |
| | Laboratory | 120 | 107–124 | 32,588 | 0 |

[a] Maximum absorbed dose.
[b] Third instars were artificially inoculated into fruit 24 h before treatment unless stated otherwise.
[c] Most tolerant species in dose–response test comparisons.
[d] Two infestation methods used: artificial inoculation, 62,400 individuals; and natural oviposition, 31,266 individuals (estimated from controls).

survivors to the adult stage, and no pupae were partially emerged. Adult emergence in controls was 68%.

## Discussion

Melon fly was the most tolerant of the three species to irradiation, and oriental fruit fly was more tolerant than Mediterranean fruit fly. Laboratory and wild strains of each species were equally tolerant of irradiation, and larvae were more tolerant when irradiated in fruit compared with artificial diet. An irradiation dose of 150 Gy (measured doses of 129–144 Gy) applied to 93,666 melon fly late third instars in papayas resulted in no survival to the adult stage (Table 5), indicating that this dose is sufficient to provide quarantine security at the probit-9 level (99.9968% mortality, minimum 93,613 individuals treated with no survivors) (Couey and Chew 1986, Robertson et al. 1994). Because melon fly was the most tolerant species, an irradiation dose of 150 Gy also will provide quarantine security for Mediterranean fruit fly and oriental fruit fly. Lowering the irradiation dose to 150 Gy for melon fly, Mediterranean fruit fly, and oriental fruit fly would reduce costs and increase capacity for treatment facilities by decreasing the required time for treatment. Irradiation doses of 100 and 125 Gy controlled Mediterranean fruit fly and oriental fruit fly, but probit 9 was not attempted.

The approved doses for Hawaii's main economic species of fruit flies—210 Gy for melon fly, 225 Gy for Mediterranean fruit fly, and 250 Gy for oriental fruit fly—suggest that melon fly is the least tolerant of irradiation and that Mediterranean fruit fly is comparable to the two *Bactrocera* spp. Our data show that melon fly is the most tolerant species and that Mediterranean fruit fly is considerably less tolerant of irradiation than either *Bactrocera* species. For example, in our study, mean mortality of laboratory strain Mediterranean fruit flies irradiated in papaya at 50 Gy was 99.93% (one adult emerged from 1500 third instars), whereas mean mortality in melon fly and oriental fruit fly irradiated at this dose was 82.9% (205 adults emerged from 1,200 third instars) and 88.3% (70 adults emerged from 600 third instars), respectively. Mansour and Franz (1996) and Bustos et al. (2004) also reported very low adult emergence in Mediterranean fruit fly third instars irradiated at 30–50 Gy in fruit.

Previous irradiation studies with tephritid fruit flies frequently had shortcomings, including inadequate sample size, failure to treat the most radiotolerant stage, irradiation of naked larvae or larvae in diet rather than in fruit, inadequate or unreported dosimetry, an insufficient number or range of irradiation doses, and incomplete or inexact reporting of the experimental methods. These shortcomings make evaluation and comparison of the results from different studies difficult and have probably led to variable predictions for doses providing quarantine security.

Burditt (1994, 1996), Rigney (1989), Nation and Burditt (1994), and Hallman and Loaharanu (2002) provided detailed reviews of the literature on fruit fly irradiation. The majority of economically important tephritid fruit flies come from four genera—*Anastrepha*, *Bactrocera*, *Ceratitis*, and *Rhagoletis.* Results with three species of *Rhagoletis* [*R. mendax* Curran, *R. pomonella* (Walsh), and *R. indifferens* Curran] suggest it is the least tolerant of these genera and can be controlled with an irradiation dose of <50 Gy. Mostly consistent results with five species of *Anastrepha* [*A. fraterculus* (Wiedemann), *A. ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann), and *A. suspensa* (Loew)] suggest an irradiation dose of 70 Gy might be sufficient to prevent emergence of adults capable of flight. Results with *C. capitata* are inconsistent, with estimated irradiation doses to control this species ranging from 50 to 500 Gy (Seo et al. 1973; Fesus et al. 1981; Kaneshiro et al. 1985; Bustos et al. 1992; Arthur et al. 1993a, b). Two studies that included large-scale testing in mangoes suggested that 150 Gy would control Mediterranean fruit fly (Bustos et al. 1992, 2004). Studies done with four species of *Bactrocera* (*B. cucurbitae* Coquillett, *B. dorsalis* Hendel, *B. jarvisi* (Tryon), and *B. tryoni* (Froggatt)] generally suggest ≤150 Gy is an efficacious dose, but the results of Seo et al. (1973) suggested a higher dose (250 Gy) was required to control *B. dorsalis.* Large-scale tests with another species, *Bactrocera latifrons*, in Hawaii indicated that 150 Gy was sufficient to prevent adult emergence in this species (T. Phillips, personal communication).

Most previous irradiation studies with tephritid fruit flies have used laboratory strains of flies. The main advantages in using laboratory flies in quarantine research is the ease with which large numbers can be reared for treatment, and control mortality in laboratory flies on diet is typically low compared with control mortality in wild flies on diet or in fruit. In our studies, control mortality of laboratory melon flies on diet was generally <10%, whereas control mortality of laboratory and wild strain melon flies treated and reared through on papaya was 20–45%. Tests using wild flies on diet were omitted from our experimental design because of inherently low control survivorship. When large numbers of flies must be tested (e.g., large-scale confirmatory tests in quarantine studies), laboratory flies should be compared with wild flies to determine whether their radiotolerance is comparable. Laboratory and wild strains were equally tolerant of irradiation in our study.

Our results showed that fruit should be used instead of artificial diet because fruit fly radiotolerance varied markedly between the two substrates (Tables 1, 3, and 4). Higher tolerance of fruit flies in fruit compared with diet may be the result of a lower oxygen concentration in fruit. Oxygen concentration is known to modify radiosensitivity and conditions producing hypoxia can increase radiation tolerance (Alpen 1998). Fruit also should be used because it most closely simulates real quarantine treatment conditions.

The generic dose concept has been applied on a limited scale to irradiation treatment for fruits exported from Hawaii to the U.S. mainland. After a rambutan shipment from Hawaii to California was interrupted in 1997 due to the presence of surface pests, the California Department of Food and Agriculture reviewed studies from Japan (Dohino et al. 1994, 1996; Kumagai and Dohino 1995) on surface pests (thrips, mites, and mealybugs) and established a generic treatment dose of 400 Gy (minimum absorbed dose) for all surface pests. In 2001, the USDA–APHIS convened a meeting to establish treatment protocols for a new commercial irradiation facility (Hawaii Pride LLC) in Hawaii and approved generic irradiation doses of 250 Gy for any species of Tephritidae (fruit flies) and Thysanoptera (thrips); and 400 Gy for any species of Coccidae (soft scales), Pseudococcidae (mealybugs), and immature Lepidoptera (moths) infesting eight fruits being exported to the U.S mainland (USDA–APHIS, unpublished document). In this case, the doses for nonfruit fly pests were established based on information from studies in Japan and Hawaii on a limited number of species within each taxa (Dohino et al. 1994, 1996, 1997; Follett and Lower 2000; Hara et al. 2001; Yalemar et al. 2001; Jacobsen and Hara 2003; IDIDAS 2003). This was the first time USDA–APHIS recommended a generic irradiation dose for any group of insects albeit on a limited scale and only for certain Hawaii fruits. New Zealand is preparing a rule to allow import of tropical fruits from Australia by using generic irradiation treatments of 150 Gy for fruit flies, 250 Gy for other insects, and 300 Gy for mites (Corcoran and Waddell 2003).

Hawaii's pest tephritid fruit flies have been an anomaly when presenting the case for a generic irradiation quarantine treatment dose of 150 Gy for Tephritidae (Hallman and Loaharanu 2002). Results of our study with melon fly, Mediterranean fruit fly, and oriental fruit fly support a proposed generic dose of 150 Gy for all tephritid fruit flies. The use of generic doses is expected to accelerate the process of approving irradiation quarantine treatments for specific crops, and thereby rapidly expand exports.

Broad application of the generic irradiation treatments to other taxa at the family or order level would be beneficial to promote trade in agricultural commodities and provide a treatment alternative for infested consignments arriving in importing countries. An International Database of Insect Disinfestation and Sterilization (IDIDAS 2003) under development by the International Atomic Energy Agency contains information on many Coleoptera (79 species, mainly curculionids) and Lepidoptera (72 species, mainly pyralids and tortricids); however, the majority of the studies referenced were not designed for quarantine purposes and lack the necessary large-scale tests. Information for other important regulatory arthropod groups such as Thysanoptera, Hemiptera, and Acari is limited. Before generic treatments can be recommended for a wider range of insects and on a broader scale, information from coordinated research projects and large-scale tests is needed on effective irradiation doses for key pests and underrepresented taxa. The most radiotolerant insect species tested to date is the Angoumois grain moth, *Sitotroga cerealella* (Olivier), which successfully reproduced at 500 Gy but not at 600 Gy (Ignatowicz 2004). Theoretically, this dose could be set as a generic treatment for all insects; however, a limiting factor for the practical use of a generic treatment at 600 Gy is the 1000 Gy (1 kGy) maximum allowed dose for fresh produce set by the Food and Drug Administration. With typical dose uniformity ratios at commercial irradiation facilities of 1.5–3.0, treatment to achieve a minimum absorbed dose of 600 Gy without exceeding 1 kGy would be difficult. Also, doses >600 Gy adversely affect the organoleptic properties of many fresh commodities (Morris and Jessup 1994).


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